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PRIMARY EVENTS IN VISION.(U)
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Primary Events in Vision.

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In this report ~~I will~~ focus on the fundamental mechanisms by which light energy is converted into a neural response in visual photoreceptor cells. These fundamental mechanisms lie at the basis of many of the visual effects we have discussed during this conference. I hope I will be able to convince you that research in this area has approached the stage that ~~allows us to start to piece~~ permits the piecing together the molecular mechanisms between absorption of light and the generation of a neural response. There is still a long way to go, but great progress is being made, and I am convinced that as a result of this progress we will be able to make a significant contribution to understanding some of the presently more difficult-to-appreciate psycho-physical measurements.

To begin with, let me start with a review of the questions that many of us in this field are trying to answer. In Figure 1 we see before us a diagram of a rod cell. These cells are responsible for black-white and dim light vision, and these are the cells which have been the focus of much of the research presently in this field. The rod cell is composed of an outer segment containing frisbee-like disc membranes in which are embedded the primary molecule in visual photoreception. These disc membranes are stacked one on top of another and are surrounded by a pl[illegible]rane. The outer segment is connected to the inner segment by a thin cillium, and the inner segment contains mitochondria, the nucleus, and ends in a synaptic terminal to the neuronal network of the retina.

In the dark there is a continual dark current of sodium ions moving through the plasma membrane of this cell. When light strikes

the primary photoreceptor molecule (which are represented as triangles in Figure 1), chemical changes are initiated in the cell which finally mediate the sodium ion conductance across the plasma membrane. This causes an imbalance of ions across the plasma membrane and causes a hyperpolarization of the plasma membrane and subsequently a neural response.

Let us now consider what we know about the primary molecule of visual photoreception--rhodopsin. ~~As can be seen~~ in Figure 2, the molecule is composed of a form of vitamin A which is called retinal, and this form of vitamin A is connected through a Shiff base-nitrogen linkage to a membrane glycoprotein matrix called opsin. The combination of retinal and opsin is called rhodopsin. Within a picosecond, i.e., 10^{-12} seconds after rhodopsin absorbs a photon, it is transformed to a red absorbing species called bathorhodopsin, which is the primary photo-product of the absorption of light. Subsequent to that process are a series of thermal steps which produce progressively bluer absorbing species, and in the final regeneration process the retinal breaks away from the opsin matrix and is regenerated enzymatically back into the rhodopsin molecule.

One of the first questions that we have to ask about this rhodopsin molecule is why it absorbs where it does. If one takes free retinal, one finds that free retinal absorbs light in the near UV around 380 nm or so, depending on the solvent. On the other hand, when the retinal is introduced into the protein matrix, it absorbs around 500 nm, as is seen in Figure 2. The cause of this red shift in the absorption of retinal, into the

primary region of the sun's emission spectrum, is due, most people believe, to the following set of arguments. In Figure 3 we find a Table which essentially tells us the dipole moment in the ground and in the excited state of the retinal molecule. As we see, the dipole moment of the retinal in the ground state, μ_G, is approximately 3.5 D, whereas the dipole moment in the excited state $\Delta\mu$, is 15.6 D. In other words, as a result of the absorption of a photon, within 10^{-14} seconds, approximately 0.21 electrons move from the ring to the nitrogen of the chain. Thus, within an extremely short period of time the charge character of the retinal changes dramatically. Now, if there were an appropriately positioned negative charge, or for that matter a positive charge, at some point along the chain [a negative charge near the ring, a positive charge near the nitrogen], one would expect that the excited state would be significantly lowered from that of the free molecule, effecting a considerable red shift in the absorption of the retinal chromophore. In fact, a protein can certainly provide such charges at appropriately positioned points, and the general feeling presently is that this is the most likely mechanism for the change in the absorption spectrum of rhodopsin from rods to cones and between species. In other words, the protein is genetically manipulated in order to produce a rhodopsin with the appropriate absorption maximum.

~~With~~ the question of ~~the~~ absorption ~~of~~ rhodopsin, ~~generally~~ believed to be answered, let us consider what transformation the light affects in the rhodopsin molecule. As we see in Figure 1, there is a large shift in the absorption maximum of rhodopsin after

photon absorption by approximately 45 nm to the red. What molecular transformation in the rhodopsin does this reflect? Let me describe to you what is possibly happening and then report on some experiments that support the more unconventional aspects of these ideas. Upon excitation of rhodopsin, as is seen in Figure 4, there is a significant charge redistribution, as we have noted earlier, and this charge redistribution initiates electrostatic interaction between the new charge distribution on the retinal and the amino acids in close contact with the retinal. These amino acids could be the ones that, in fact, interact with the retinal to give the color to rhodopsin, or they could represent some other amino acids in the neighboring environment that feel the new electrostatic charge redistribution in the chromophore. In any event, as a result of these new interactions that are initiated within 10^{-14} seconds of excitation, a proton begins to move from one R group (one amino acid) to another. This alters the charge in the neighboring environment of the retinal and stablizes the new charge distribution in the retinal chromophore. Subsequently to this there is considerable tortional movement of the retinal chromophore to move from the excited state surface onto the ground state surface. In the ground state surface there is further movement of the protons and separation of the charge groups and further retinal structural alteration to stablize the new protein conformation that is now the bathorhodopsin state. These ideas fit very well into what is known about the control mechanisms of the color of such pigments as we have noticed, because, as we have seen, in the bathorhodopsin state, the protein

has an altered amino acid retinal interaction, and this could readily account for the red shift in the bathorhodopsin state.

It has been generally accepted that retinal interaction indeed undergoes structural alteration between rhodopsin and bathorhodopsin. A new aspect represented by the above mechanism is a movement of the proton in the protein. What evidence do we have for this new aspect of the mechanism? The evidence comes from two types of measurements. One type of measurement is called picosecond spectroscopy, and basically measures on a picosecond timescale the alteration in the absorption from rhodopsin to bathorhodopsin. Such experiments have been done since the early 1970's. However, recently, Peter Rentzepis and his group at Bell Telephone Laboratories have repeated their earlier measurements on the onset of bathorhodopsin as a function of suspending the rhodopsin in deuterated media. Basically, when rhodopsin is resuspended from its natural H_2O environment into a D_2O environment, the protons in the protein which are exchangeable exchange with the deuteron from the surrounding median. There are, of course, many protons which are non-exchangeable, and this can best be seen by referring to the retinal molecule. In the retinal molecule, for example, which is embedded in the protein, all of the protons are non-exchangeable except the proton on the nitrogen which can exchange for a deuteron. Rentzepis and his colleagues, including Meredith Applebury and Kevin Peters, noticed that when they collected data for the production time of bathorhodopsin in deuterated media, the production time had been extended by approximately 7 times. This indicated that some exchangeable proton, which was now a deuteron, changed

its position in going from rhodopsin to bathorhodopsin. Naturally, they decided that since the retinal nitrogen proton (known as the Schiff base proton) was the only exchangeable proton on the chromophore, and since the retinal is the molecular entity in the protein that absorbs the light, it was logical to assume that the proton changing its position must be the proton on the Schiff base nitrogen. And this was the starting point of a molecular theory which they've proposed for the primary events in vision. However, there is a second experimental technique that can be brought to bare on this issue. That technique is called resonance Raman scattering. Resonance Raman scattering can selectively look at the vibrational spectrum of the absorbing chromophore. In other words, resonance Raman scattering can look at the vibration of simply the retinal above the surrounding background of the protein, the lipid and the hundreds and hundreds of other molecules in the cell. Using this technique, one can clearly identify the vibrational mode which corresponds to the carbon nitrogen bond. This vibrational mode is a carbon nitrogen stretching frequency and occurs at approximately 1655 cm^{-1}. If indeed the hypothesis of Rentzepis, Applebury and co-workers is correct, i.e., that the hydrogen on the Schiff base is the one responsible for the large deuterium isotope effect discovered in their picosecond measurements, then, one should be able to monitor the vibrational spectrum of the carbon nitrogen stretch as one increases the concentration of the batho intermediate. This would result in changes in the C=N vibrational frequency change as the batho intermediate is increased in concentration. The above arises directly from the hypothesis that the Schiff base proton

changes its position, and this would necessitate a change in electron density in the C=N bond, resulting in an altered carbon nitrogen stretching frequency. The resonance Raman experiment that we have performed that corresponds to this experimental situation is seen in Figure 5. In this figure the top spectrum contains a large concentration of the batho intermediate, and as is labelled in the figure, the C=N⊕H stretch is clearly observed. Those bands marked B in the spectrum corresponds to the batho intermediate. If one monitors the Raman spectrum with the same laser line as the top spectrum in this figure, i.e., 482.5 nm, and simultaneously illuminates the sample with a laser at 580.0 nm, one sees a reduced concentration of the batho intermediate. This reduction in batho concentration is clearly seen by the decrease in the 1537 wave number band in going from the top spectrum to the bottom spectrum, the bottom spectrum being the one which has both laser illuminations on the sample. The thing that is clear in the comparison of these two spectra is that even though the band at 1537, which is associated with the batho intermediate as is seen by its significant decrease with the addition of the 580.0 nm laser line, the 1655 CNH stretch is totally unchanged, demonstrating that the proton that is responsible for the deuterium isotope effect in the picosecond experiment cannot be the one associated with the Schiff base. Furthermore, since the Schiff base proton is the only exchangeable proton on the chromophore, the protons that are moving in the picosecond experiments must be associated with some other amino acid residues in the protein. Therefore, going back to Figure 4 we can now review the steps that take place in visual

excitation. The absorption of a photon causes significant charge redistribution in the retinal chromophore leading to the P* state in the figure, that induces proton movement from one amino acid to another, possibly even beginning the motion of separation of the protein residues. Then, there is significant tortional oscillation at Px which brings the molecules back to the ground state where there is further proton movement, further protein structural alteration and further retinal structural alteration which stabilizes the new protein structure of bathorhodopsin.

The significance of these measurements indicates that, as a result of the absorption of light, one of the first molecular events is the motion of a proton, and this is probably the first ion movement that is initiated by the rhodopsin molecule. ~~In addition to this proton,~~ other experiments are showing that there are several other chemical species activated by the absorption of light in the rhodopsin molecules. One of these species, calcium ions, has been known for many years through the experiments of Hagins, Yoshikami and co-workers at the National Institutes of Health. Other species, such as the enzymes GTPase, are also light activated. Furthermore, the enzyme phosphodiesterase is also known to be activated by the absorption of a photon by the rhodopsin molecules. Basically then, several new actors have entered the stage of visual transduction, and the experiments done under this contract during the past year have been responsible at singling out one of the first ionic species to be moved as a result of light absorption. A variety of new experiments are presently being performed in our laboratory to try and ellucidate the exact sequence of

events from the absorption of a photon to the generation of a neural response. Some of these experiments include the discovery that rhodopsin molecules in the disc membrane may be extremely sensitive, in terms of their absorption properties, to any disc membrane potential that may be initiated as a result of ion transport. This is indicated by experiments described at the contractors' meeting, where I was able to show that rhodopsin incorporated in lipid bilayers clearly had absorption properties that could be altered as a function of imbalances in ionic concentration across the membrane. In addition to these experiments, we are in the process of trying to develop a light probe that would be spacially sensitive on an angstrom scale. Such a light probe will be used to further define the spacial association of the enzyme's phosphodiesterase and GTPase to the rhodopsin molecules that initiate their reaction. At this point, one thing is clear--the situation in the field of the molecular events of visual transduction is at the stage where many of the primary actors are probably known. What remains is to arrange the molecular events in their appropriate order. When this happens during the next few years, we will be in a position to alter significantely visual functions by clearly knowing the molecular events that control neural response generation. In terms of practical problems associated with visual response, this will initiate a new era where the molecular events will be able to be connected directly to many of the neurological and psychophysical phenomena that are of interest to the United States Navy.

Figure 1: Diagram of a rod cell.

Figure 2: Rhodopsin and its bleaching sequence.

Figure 3: Dipole moments of retinal.

Figure 4: The mechanism of visual excitation.

Figure 5: Analysis of the C=N vibrational frequency.

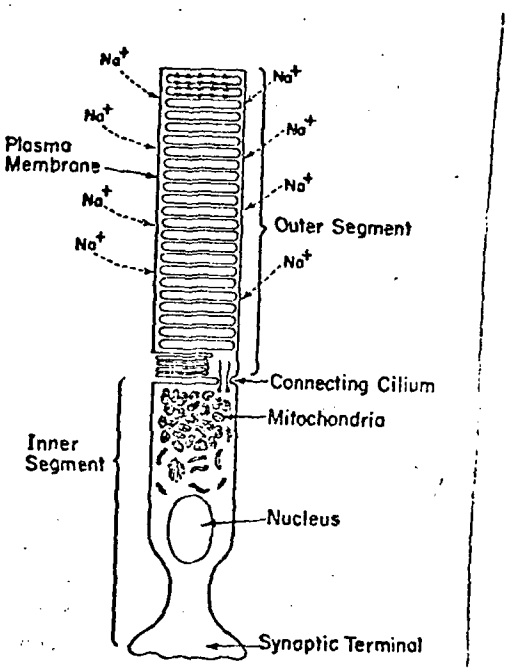
Na+
Plasma Membrane
Outer Segment
Connecting Cilium
Mitochondria
Inner Segment
Nucleus
Synaptic Terminal

Fig. 1

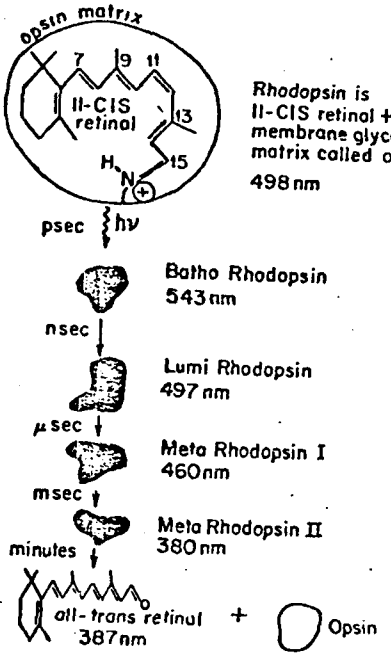
opsin matrix
7
9
11
11-CIS
retinal
13
H
N
15
Rhodopsin is
11-CIS retinal + a
membrane glycoprotein
matrix called opsin
498 nm
psec
hν
Batho Rhodopsin
543 nm
nsec
Lumi Rhodopsin
497 nm
μsec
Meta Rhodopsin I
460 nm
msec
Meta Rhodopsin II
380 nm
minutes
all-trans retinal
387 nm
+
Opsin

Fig. 2

all-trans retinal

$\lvert\Delta\mu\rvert$	15.6 ± 1 D*	
$\lvert\Delta\mu \cdot \mu_G\rvert$	620 ± 1.3 D	* Mathies et al, PNAS, 73, 2169 (1976).
$\lvert\mu_G\rvert$	3.5 ± 3.3 D	
$\lvert\hat{p} \cdot \Delta\mu\rvert$	14.7 ± 2.1 D	
$\lvert\hat{p} \cdot \mu_G\rvert$	4.3 ± 0.8 D	

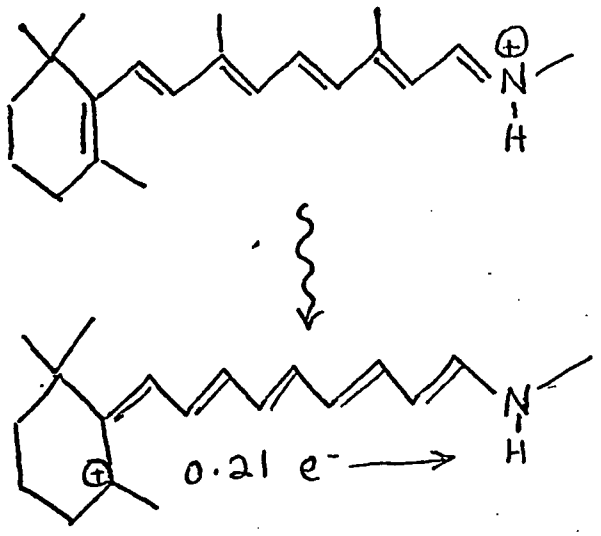

Fig. 3

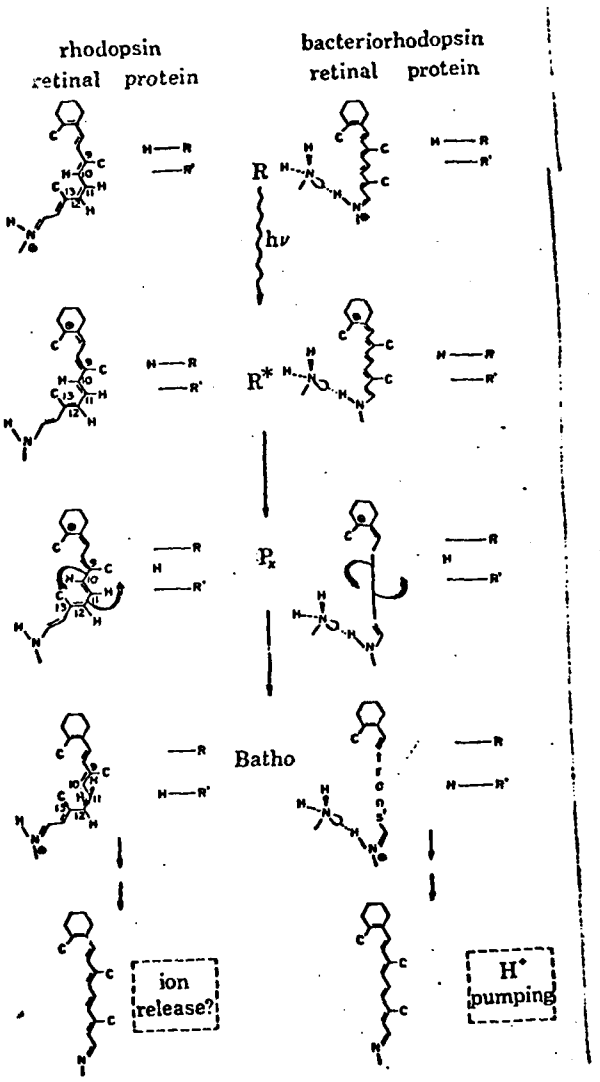
rhodopsin
retinal protein
bacteriorhodopsin
retinal protein
hν
R*
P
Batho
ion release?
H+ pumping

Fig. 4

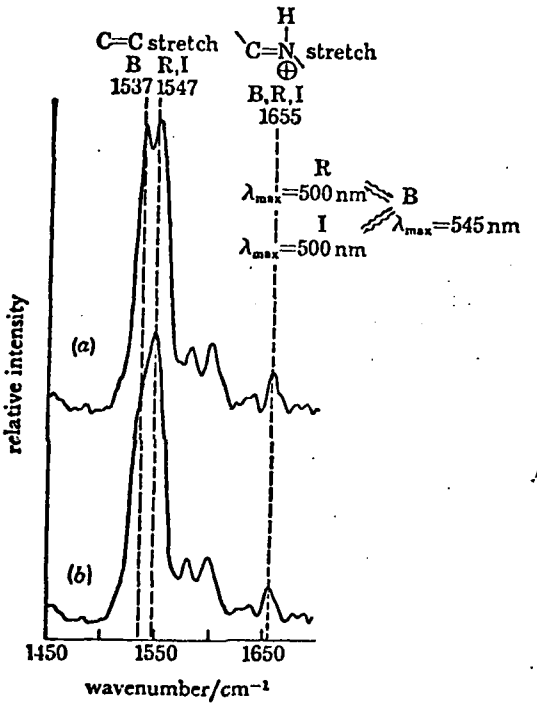
C=C stretch
B R,I
1537 1547
H
C=N stretch
⊕
B,R,I
1655
R
λmax=500 nm
B
I
λmax=545 nm
λmax=500 nm
(a)
(b)
relative intensity
1450
1550
1650
wavenumber/cm⁻¹

Fig. 5


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